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In Vitro Oral Biofilm Formation on Triclosan-Coated Sutures in the Absence and Presence of Additional Antiplaque Treatment

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Purpose: This study evaluated the in vitro plaque inhibitory effect of triclosan-coated polyglactin 910 sutures in the absence and presence of an additional antiplaque agent commonly used after oral surgery.

Materials and Methods: Triclosan-coated sutures were incubated for 4 hours in freshly collected human saliva and, when appropriate, subsequently treated with an antiplaque rinse containing chlorhexidine-cetyl pyridinium as active components. Sutures without a triclosan-coating served as a control.

Results: Triclosan-coated sutures harbored similar amounts of plaque as did uncoated sutures. Exposure to the antiplaque rinse caused significant decreases in viable organisms for uncoated and triclosan-coated sutures. However, after application of the antiplaque rinse, more micro-organisms were found on triclosan-coated than on uncoated sutures.

Conclusion: Sutures coated with triclosan do not provide a sufficient antimicrobial effect to prevent in vitro colonization by oral bacteria, whereas use in combination with a chlorhexidine-cetyl pyridinium-containing antiplaque rinse appears to be counterproductive.

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Sutures are used frequently in oral surgery, for instance, after the surgical removal of third molars, implant surgery, and a variety of periodontal procedures. Sutures used in the oral cavity, however, become rapidly covered with dental plaque or biofilm, providing a reservoir of pathogens that may cause inflammation of neighboring tissue. The incidence of infections after third molar surgery is 1% to 5% and the reported overall prevalence of periodontal post-surgical infections varies from less than 1% to 5.4%.¹⁻⁴

In general surgery, skin-derived *Staphylococcus aureus* and *Enterococcus* sp are held responsible for most postsurgical wound infections.^{5,6} However, the oral microflora contains many other specific opportunistic pathogens, such as streptococci, *Bacteroides*, *Fusobacterium*, *Prevotella*, and *Porphyromonas*, which may hamper oral wound healing^{7,8} or act as a focus for postsurgical, odontogenic infections.^{9,10}

Postsurgical infections are accompanied by high costs and patients' inconvenience, because they often

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require additional surgery, extended hospitalization, and use of antibiotics or other oral antimicrobials. In general, however, once bacteria are in a biofilm mode of growth, their susceptibility to antibiotics and other antimicrobials is low. Postsurgical care regimens typically involve chlorhexidine rinsing to avoid plaque formation,^{11,12} although the efficacy of chlorhexidine strongly decreases when a biofilm matures.¹³ Triclosan is another antimicrobial agent widely used in personal care and hygiene products such as deodorants and soaps and in oral health products to decrease plaque formation and treat inflammatory lesions.^{14,15} Recently, triclosan-coated polyglactin 910 sutures have been introduced and demonstrated to be effective in preventing suture-colonization by wild-type and methicillin-resistant *S aureus* and *Staphylococcus epidermidis*.¹⁶

The aim of the present work was to determine the efficacy of triclosan-coated sutures on oral biofilm formation in vitro in the absence and presence of the additional application of a chlorhexidine-cetyl pyridinium-containing antiplaque rinse.

Materials and Methods

SUTURE MATERIALS

Sterile polyglactin 910 sutures (size 3-0) were used with and without a triclosan (2,2,4-trichloro-2-hydroxy-diphenyl ether) coating, ie, Vicryl Plus Antibacterial and Vicryl (Johnson & Johnson Medical BV, Amersfoort, The Netherlands). Both sutures are absorbable, braided, and violet colored and were obtained in sterile packages. Vicryl Plus Antibacterial contains triclosan up to 150 µg/m. Control experiments were performed to check the antibacterial efficacy of the triclosan-coated sutures used by measuring the zone of inhibition around a streptococcal and staphylococcal strain. To this end, *Streptococcus sanguis* PK1889 and *S aureus* O⁻ were precultured from blood agar in 10 mL of Todd Hewitt Broth (Oxoid, Basingstoke, UK) or Tryptone Soya Broth (Oxoid), respectively, for 24 hours at 37°C in ambient air. From each preculture, blood agar plates were prepared. After placing 1 length of a triclosan-coated suture on the inoculated blood agar plates, plates were incubated at 37°C for 48 hours in ambient air. Subsequently, the plates were scanned for a zone of inhibition around the triclosan-coated suture. In addition, blood agar plates were inoculated with human whole saliva to observe possible zones of inhibition around the sutures for the mixed, total salivary microflora (for saliva collection, see below).

ANTIPLAQUE RINSE

Perio-aid (Dentaïd BeNeLux BV, Houten, The Netherlands), containing 0.12% chlorhexidine, 0.05% cetyl

pyridinium chloride, and no alcohol, was used as an antiplaque rinse.

COLLECTION AND MICROBIAL COMPOSITION OF SALIVA

Fresh human whole saliva was used as an inoculum for biofilm formation on the sutures and was obtained from a pool of 28 healthy female and male donors. Saliva was collected into ice-chilled cups, after stimulation of the salivary flow by chewing Parafilm (Pechiney Plastic Packaging, Chicago, IL), in line with the rules set out by the medical ethical committee of the University Medical Center Groningen and the tenets of the Declaration of Helsinki and with the informed consent of the donors. After collection and pooling, phenylmethylsulfonyl fluoride (0.2 mol/L; Merck, Darmstadt, Germany) was added to a final concentration of 1 mmol/L as a protease inhibitor. Subsequently, the microbial composition of the pooled saliva was determined by dilution plating. To determine the total number of cultivable microorganisms in saliva, dilution plating was done on sheep blood agar (50 mL/L) supplemented with hemin (5 mL/L) and menadione (1 mL/L) and incubated for 14 days under anaerobic conditions at 37°C. The following selective plates were used: TYS20B agar¹⁷ for *Streptococcus mutans*, which was incubated for 5 days anaerobically at 37°C followed by 2 days aerobically at 20°C; MSB agar¹⁸ for *Streptococcus mitis* and *Streptococcus salivarius*, which was incubated for 2 days aerobically; and MRS agar (Merck) for lactobacilli, which was incubated for 2 days with 5% CO₂. After incubation, colony-forming units (CFUs) were counted. Nonagreeable species to the selective agar plates were excluded based on morphology by microscopic analysis.

BIOFILM FORMATION ON SUTURES

Sterile test tubes were filled with 60 mL of fresh human saliva and sealed with cotton wool. Two lengths (5 cm each) of triclosan-coated and uncoated sutures were put into the saliva through the cotton wool until the sutures were totally submerged. Incubation was done for 4 hours while shaking (New Brunswick Scientific Classic 24, Edison, NJ) at 37°C and 60 rpm to simulate saliva flow around the sutures. After incubation, all sutures were rinsed in sterile reduced transport fluid (RTF).¹⁹ One of the 2 lengths was put in a sterile cup with 1.5 mL of sterile RTF. The other length was immersed in 15 mL of the antiplaque rinse and hand-shaken for 30 seconds. Subsequently, this length was rinsed in sterile RTF and put in a cup with 1.5 mL of sterile RTF. Biofilm analysis was carried out within 15 minutes.

Table 1. NUMBER OF VIABLE MICRO-ORGANISMS IN SALIVA

Bacterial Group	Mean Log ₁₀ CFU/mL
<i>Streptococcus mutans</i>	6.8
<i>Streptococcus mitis</i> / <i>Streptococcus salivarius</i>	6.6
<i>Lactobacilli</i>	6.8
Total number of micro-organisms	7.9

Abbreviation: CFU, colony-forming unit.

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BIOFILM ANALYSIS

Selective plate counting was applied to enumerate the number of viable organisms on the sutures, and intact biofilms on the sutures were examined using confocal laser scanning microscopy (CLSM). For selective plate counting, adhering bacteria were detached from the sutures by sonication in RTF for 30 seconds (3 times 10 seconds with a 20-second interval) at 30 W, 90% duty cycle, and a microtip output limit of 2.5 (Vibra Cell model 375; Sonics and Materials Inc, Danbury, CT) while cooling in an ice-water bath. Subsequently, the resulting bacterial suspensions were dilution plated, as described above. Average CFUs were calculated for all types of agar plates and converted to log values per centimeter of suture

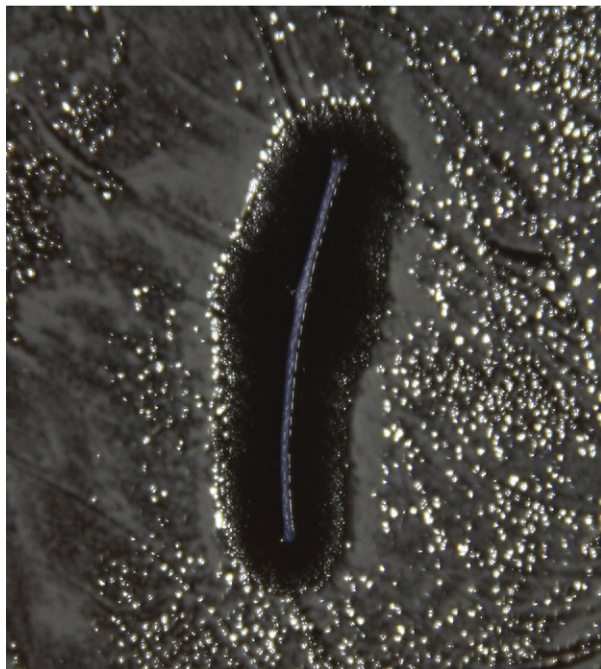


FIGURE 1. Presence of a zone of inhibition around a triclosan-coated suture against triclosan-sensitive *Staphylococcus aureus* O⁻.

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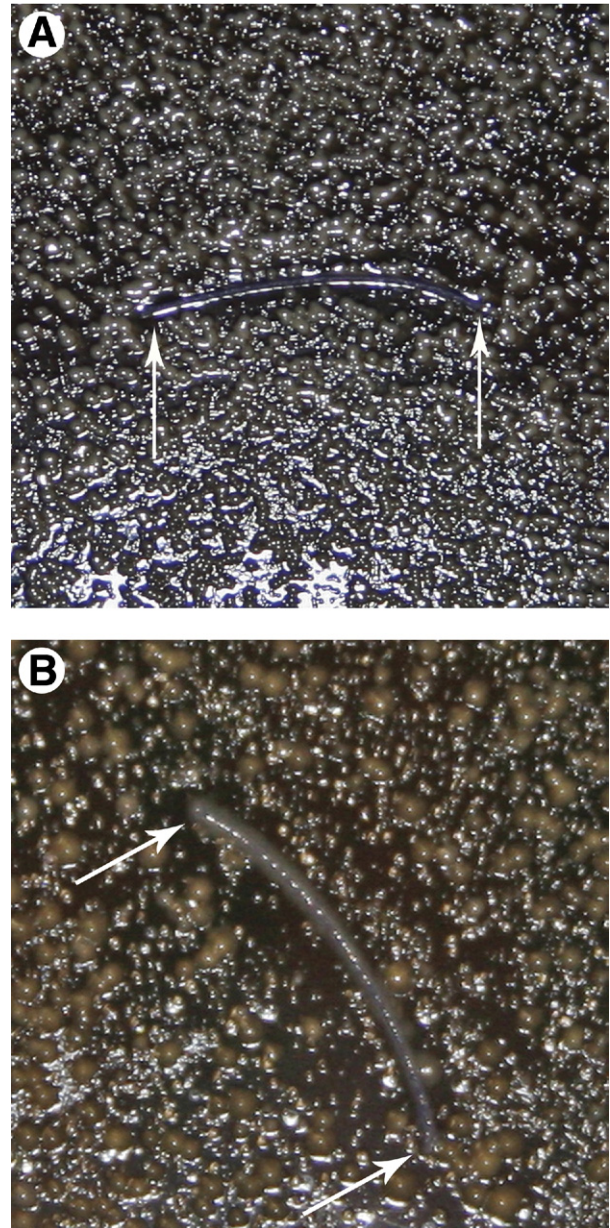


FIGURE 2. Absence of zones of inhibition around triclosan-coated sutures against *Streptococcus sanguis* PK1889 (A) and total salivary microflora (B). B, Note that oral microflora has excreted a slimy layer over the suture. Arrowheads indicate the tips of the suture.

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length. CLSM was applied to check whether sonication had indeed removed all bacteria from the suture.

All quantitative analyses were carried out at least in triplicate. The statistical significance of differences between 2 means was examined by paired Student's *t* test, and *P* less than .05 was considered a significant statistical difference.

For CLSM analysis of the biofilms, sutures with intact biofilms were stained with a LIVE/DEAD

Table 2. NUMBER OF VIABLE ORGANISMS (LOG₁₀ UNITS PER CENTIMETER SUTURE) IN BIOFILMS ON SUTURES WITH AND WITHOUT TRICLOSAN COATING IN THE ABSENCE AND PRESENCE OF AN ADDITIONAL ANTIPLAQUE RINSE

Bacterial Group	Without Triclosan Coating		With Triclosan Coating	
	–APR	+APR	–APR	+APR
<i>Streptococcus mutans</i>	4.1 ± 0.5	ND*†‡	4.5 ± 0.4	1.6 ± 0.6*
<i>Streptococcus mitis</i> / <i>Streptococcus salivarius</i>	4.4 ± 0.3	ND*†‡	4.2 ± 0.5	1.3 ± 1.3*
<i>Lactobacilli</i>	4.0 ± 0.4	ND*†‡	4.5 ± 0.6	0.7 ± 0.8*
Total number of micro-organisms	5.9 ± 0.1	1.6 ± 1.4*†‡	6.1 ± 0.5	3.1 ± 0.8*

Abbreviations: –APR, absence of additional antiplaque rinse; +APR, presence of additional antiplaque rinse; ND, no organisms detected.

Values for specific bacterial groups are presented as mean ± SD over 3 separate experiments, and the total number of micro-organisms was determined in 5 separate experiments.

* $P < .05$ versus –APR in the absence and presence of a triclosan coating.

† $P < .05$ versus with triclosan coating and –APR.

‡ $P < .05$ versus with triclosan coating and +APR.

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Baclight Bacterial Viability Kit (Molecular Probes, Leiden, The Netherlands). This stain contains SYTO9 dye (3.34 mmol/L) and propidium iodide (20 mmol/L). The CLSM used was a Leica TCS SP2 (Leica Microsystems Heidelberg GmbH, Heidelberg, Germany), equipped with a 20× oil immersion objective. Emission wavelengths were 500 to 600 nm at an excitation wavelength of 488 nm for detection of the green fluorescent SYTO9 and 560 to 700 nm at an excitation wavelength of 543 nm for detection of the red fluorescent propidium iodide. Viable bacteria exhibit green fluorescence, whereas dead bacteria exhibit red fluorescence. Representative images were taken of each suture by scanning series of about 40 images (with steps of 3 μm) and stacked into overlay projections.

Results

The concentrations in saliva of the different bacterial groups are presented in Table 1. During the experimental period, bacterial concentrations of the pooled saliva were regularly checked and found to coincide within 0.1 log₁₀ CFU/mL. The control experiment for *S aureus* O[–] was positive (Fig 1) and a zone of inhibition with a diameter of 6 mm was observed. In addition, no zones of inhibition were observed for *S sanguis* PK1889 (Fig 2A) and the total salivary microflora (Fig 2B).

There was no significant difference in the number of CFUs found on sutures with or without a triclosan coating (Table 2), in line with the absence of zones of inhibition around triclosan-coated sutures on plates inoculated with streptococci or total salivary microflora (Fig 2). On both types of sutures, the number of CFUs for all bacterial groups decreased significantly ($P < .05$, paired Student's *t* test) after additional antiplaque treatment. However, use of a chlorhexidine-cetyl

pyridinium-containing antiplaque rinse on biofilms formed on triclosan-coated sutures yielded more viable organisms than on biofilms formed on uncoated sutures ($P < .05$, paired Student's *t* test).

Numbers of green fluorescent, live bacteria on sutures without and with triclosan-coating were similar (compare Figs 3A,B, respectively), in correspondence with the data listed in Table 2. Use of the chlorhexidine-cetyl pyridinium-containing antiplaque rinse yielded an increase in red fluorescence for both types of sutures (Figs 3C,D), indicating a decrease in the number of viable bacteria.

Discussion

Biofilm formation on oral sutures can act as a focus of infection and form a potential risk for wound healing after oral surgery. Good sutures require specific physical characteristics and properties to limit or prevent bacterial colonization of parts exposed to saliva. Currently, different types of synthetic sutures are used in oral surgery, monofilament versus multifilament and resorbable versus nonresorbable. In general, monofilament and resorbable sutures show less biofilm formation than multifilament and nonresorbable sutures.^{20–22} A recent effort aimed at further decreasing suture-mediated infections by triclosan coating of polyglactin 910 sutures.

Triclosan, a phenyl ether, is predominantly bacteriostatic. At low concentrations the growth of many gram-positive and gram-negative nonsporulating bacteria is inhibited.¹⁵ Its spectrum includes minimum inhibitory concentration (MIC) values in the order of lower than 0.001 to 7.8 μg/mL for a range of oral bacterial strains cultured in broth. However, these MIC values increase when protein is added to the growth media.²³ Above these MICs, triclosan becomes rapidly bactericidal.¹⁵

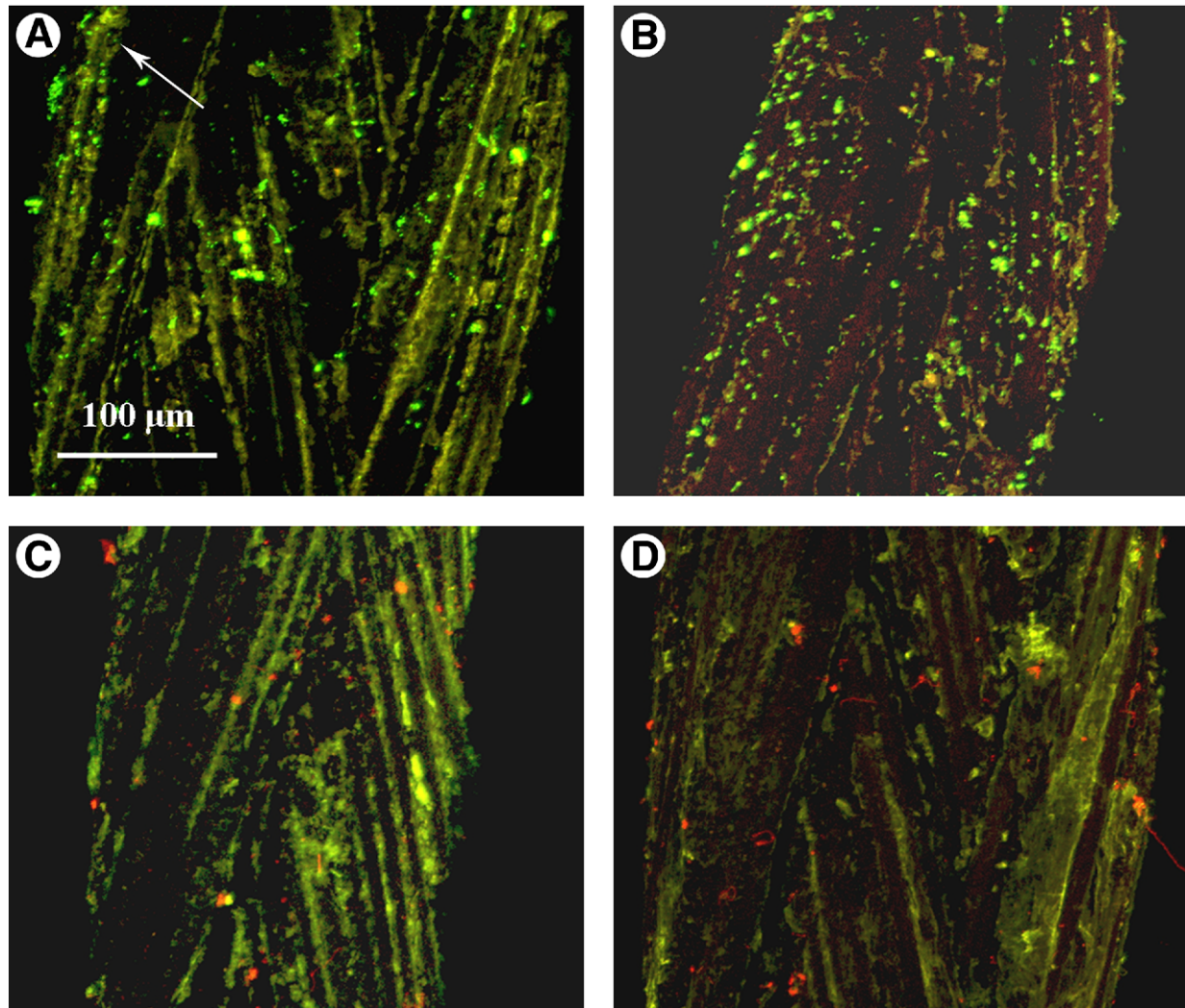


FIGURE 3. Examples of CLSM images from sutures without (A, C) and with (B, D) a triclosan coating before (A, B) and after (C, D) exposure to chlorhexidine-cetyl pyridinium-containing antiplaque rinse. Fluorescent green represents viable bacteria (arrow in A); red represents nonviable bacteria. Note that the color of the suture filaments itself varies from olive green (A, C, D) to brown (B).

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Triclosan is formulated into toothpastes and mouth-rinses in concentrations up to 0.3% (3 mg/g) and 0.03% (0.3 mg/mL), respectively. This is much higher than the cited MIC values. However, in vivo, the antiplaque efficacy of triclosan alone is limited^{24,25} due to lack of substantivity. Oral retention of triclosan has been increased through the inclusion of polyvinyl methyl ether maleic acid. Furthermore, its antimicrobial effect has been increased in oral health care products through the addition of zinc citrate.²⁶⁻²⁹

The antimicrobial effect of triclosan-coated sutures has been shown only for nonoral, single species, such as *S aureus*, *S epidermidis*, and *Escherichia coli*,^{16,30} but its use in oral health care products suggests benefits of the use of triclosan-coated sutures in oral surgery. Therefore, the total lack of efficacy of a triclosan coating on oral biofilm formation (Figs 3A,B; Table 2)

was surprising. Although the efficacy of the triclosan-coated sutures against a triclosan-sensitive *S aureus* O⁻ strain was confirmed (Fig 1), the triclosan-coated sutures yielded no measurable zones of inhibition against the oral bacteria (Figs 2A,B). This is in line with suggestions that antimicrobial effects of triclosan-containing toothpastes and mouth rinses are not solely due to triclosan, but rather to its combination with polyvinyl methyl ether maleic acid or zinc citrate triclosan.²⁶⁻²⁹

Chlorhexidine, a cationic agent with hydrophilic and hydrophobic properties, is still the leading antimicrobial to combat supragingival and mucosal plaques. It is often prescribed after oral surgery, when mechanical plaque control is limited or impossible. Combination of chlorhexidine with other antimicrobials might at first instance look tempting, but clinical practice has

proved otherwise. Combination of chlorhexidine with anionic sodium lauryl sulfate, a detergent used in many dentifrices, has been demonstrated to cause inactivation of chlorhexidine.³¹⁻³³ In this study, use of a chlorhexidine-cetyl pyridinium antiplaque rinse on oral biofilms formed on triclosan-coated sutures also appeared to be disadvantageous. This may have major clinical consequences, because more bacteria survived the combination than found on uncoated control sutures (Figs 3C,D and Table 2). In contrast to sodium lauryl sulfate, triclosan is nonionic, and electrostatic interactions between chlorhexidine and triclosan probably do not form the basis for this inactivation. Possibly, hydrophobic interactions between the cationic chlorhexidine and cetyl pyridinium with triclosan molecules, possessing hydrophilic and hydrophobic properties, form the basis for the inactivation observed.

Summarizing, the present results indicate that the use of triclosan-coated sutures yields no benefits in the control of oral biofilms. Moreover, its use in combination with cationic antiplaque agents, such as chlorhexidine and cetyl pyridinium, as is common practice in oral surgery, seems to be counter-indicated when triclosan-coated sutures are applied.

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